Phytotoxic activity of clomeprop in soil and concentration of its hydrolysed metabolite DMPA in soil water

Katsuichiro Kobayashi,* Yoshihiko Tsukasaki, Suthep Tongma and Ie Sung Shim

Institute of Applied Biochemistry, University of Tsukuba, Tsukuba 305-8572, Japan

Abstract: The relationship between the fate of clomeprop in soil and its phytotoxic activity on the growth of radish (*Raphanus sativus*) seedlings was investigated in the laboratory. The phytotoxic activity of clomeprop in sea sand was much higher than in non-autoclaved soil, and the phytotoxic activity in non-autoclaved soil was higher than in autoclaved soil. The phytotoxic activity of 2-(2,4-dichloro-3-methylphenoxy)propionic acid (DMPA), a hydrolysed metabolite of clomeprop, was higher than that of the latter under both soil conditions. Clomeprop was adsorbed on soil to a greater extent than DMPA. The concentration of clomeprop in soil water of non-autoclaved soil decreased with increase of the DMPA concentration in the soil water in a time-dependent manner. It is suggested that the phytotoxic activity of clomeprop applied to soil is induced mostly by the DMPA concentration in soil water after hydrolytic degradation by soil microbes.

Keywords: clomeprop; phytotoxic activity; adsorption; soil; soil water; DMPA

1 INTRODUCTION

Clomeprop[2-(2,4 -dichloro -3 -methylphenoxy)pro - pionanilide has a highly selective herbicidal activity on broad-leaved and cyperaceous weeds in rice paddy fields.[1] In the plant, clomeprop is hydrolysed at the acylamide bond to form an acid metabolite 2-(2,4-dichloro-3-methylphenoxy)propionic acid (DMPA).[2] The phytotoxic activity of clomeprop is due to the auxinic activity of DMPA[1,3] in the same way as that of naproanilide [2-(2-naphthyloxy)propionanilide].[4–6] Based on numerous studies on the fate and activity of herbicides on plant growth in soil, it was suggested that the fate and phytotoxic activity are influenced by the soil characteristics. However, there is only limited information on the relationship between the herbicidal activity and the fate of herbicide in soil.[7–9] In paddy soil, it was reported that clomeprop was metabolized to DMPA in a manner similar to naproanilide[1] but the relationship between the fate of clomeprop in soil and its phytotoxic activity was poorly documented. In previous studies,[10–13] we reported that the phytotoxic activity of thenylchlor[2-chloro-*N*-(3-methoxy-2-thenyl)-2′,6′-dimethylacetanilide] and mefenacet [2-(1,3-benzothiazol-2-yloxy)-*N*-methylacetanilide] applied to soil was well correlated with the concentration in soil water but not with the total amount in herbicide-treated soil. We suggested that the concentration of the chemicals in soil water is the most important parameter for the determination of their phytotoxic activity in soil.

This study was undertaken to clarify the relationship between phytotoxic activity of clomeprop applied to soil and its fate in soil, with emphasis placed on the concentration in soil water.

2 MATERIALS AND METHODS

2.1 Soil and application of clomeprop and DMPA

Soil was collected from paddy fields located at Ryugasaki, Ibaraki prefecture, Japan. Bulk soil samples were air-dried, ground, and sieved over a 2-mm mesh. Two hundred grams of air-dried soil (sandy loam, 0.63% total carbon, 0.06% total nitrogen, pH 6.8 (H_2O), CEC, 9.2 meq $100\,g^{-1}$) were added to 500 ml of an aqueous solution of clomeprop or DMPA containing methanol (10 ml $litre^{-1}$) at various designated concentrations, and stirred vigorously in a beaker. The soil was transferred to a plastic pot with holes at the bottom (5 cm diameter, 4 cm height), and allowed to stand for 3 h to reach the water-holding capacity by removing gravitation water. The soil was prepared for bioassay and determination of the chemicals. This soil is hereinafter referred to as applied-soil.

* Correspondence to: Katsuichiro Kobayashi, Institute of Applied Biochemistry, University of Tsukuba, Tsukuba 305-8572, Japan.

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2.2 Plant materials and bioassay

Radish (*Raphanus sativus* L var *radicula* DC cv Comet) was used as a test plant because of its high sensitivity to clomeprop and DMPA.[3] Radish seeds were surface sterilized in sodium hypochlorite solution (10 ml litre^{-1}) for 10 min, washed with autoclaved distilled water, and germinated in a Petri dish at 25°C for 24 h in the dark. Bioassay was conducted under autoclaved and non-autoclaved conditions. The former was obtained by autoclaving at 120°C for 20 min three times at 24-h intervals. Three germinated seeds were placed in the sea sand and the soil containing clomeprop or DMPA with methanol at various designated concentrations. The pots were placed in a growth chamber (light; 25°C, 14 h, 400 μE m^{-2} s^{-1}, dark; 20°C, 10 h) for five days and the shoot length of the radish seedlings was measured. Treatment was carried out with three replicates.

2.3 Adsorption on soil

Adsorption of clomeprop and DMPA on soil was determined by a batch equilibrium technique according to the method of Kuwatsuka.[14] Five grams of air-dried soil was added to 100 ml of clomeprop or DMPA solution prepared as described above and shaken at room temperature on a rotary shaker. The apparent adsorption equilibration of clomeprop was 6 h in Ryugasaki soil and the equilibration of DMPA was conveniently determined at 6 h because the adsorption of DMPA on soil was too limited and too slow to reach the equilibration time. After shaking, 5 ml of supernatant was centrifuged at 3000*g* for 20 min. The amount of clomeprop or DMPA in the supernatant was determined by HPLC, as described in Section 2.5. The difference between the initial and equilibrium concentration was considered to correspond to the amount of clomeprop or DMPA adsorbed on soil. Treatment was carried out with three replicates.

2.4 Degradation in soil

Four hundred grams of air-dried soil were poured into an aqueous solution of clomeprop or DMPA (10 μM; 1 litre) containing methanol (10 ml litre^{-1}) and stirred vigorously in a beaker. After 1 h, the supernatant in the beaker was pipetted off and the soil was placed in an inner centrifugation tube, as described in Section 2.5, and allowed to stand for 5 h to reach the water-holding capacity by removing gravitational water. The soil was placed in the growth chamber and sampled at several time intervals for the determination of clomeprop and DMPA. Treatment was carried out with three replications.

2.5 Extraction and determination of clomeprop and DMPA

The 'applied-soil' in the inner tube in a set of two tubes was centrifuged at 13 000*g* for 30 min to separate it into soil water collected in the bottom of the outer tube and the soil remaining in the inner tube,[10] referred to as 'centrifuged-soil'. After centrifugation, the soil water was immediately prepared for HPLC. Clomeprop and DMPA in the centrifuged-soil were extracted with acetonitrile + water (80 + 20 by volume). Clomeprop and DMPA concentrations were determined by HPLC using LC 10A series (Shimazu; Kyoto, Japan) equipped with auto-injector system, a reverse-phase Inertsil C 8 column (4.6 × 250 mm, particle size 5 μm; GL Science, Tokyo, Japan) and Shimazu UV spectrophotometer detector operating at 234 nm. The mobile phase consisted of a methanol + acetate buffer solution (3 + 1 by volume) at a flow rate of 0.75 ml min^{-1}. The retention times of clomeprop and DMPA were 21.3 and 10.9 min, respectively. The detectable limit was 0.02 μM and the recovery was exceeded 90%. The amount of clomeprop and DMPA adsorbed on the soil solid phase in the centrifuged-soil was calculated as follows:

$$S = (Q_{ext} - Q_{cent})/W$$

where S is the amount of adsorbed (nmol g^{-1} soil solid), Q_{ext} is the amount (nmol) in the centrifuged-soil, Q_{cent} is the amount in water remaining in the centrifuged-soil, and W is the dry weight of soil (g); the concentration in water in the centrifuged-soil was considered to be the same as in the soil water in the outer tube.[12] Treatment was carried out with three replications.

RESULTS

3.1 Effect of clomeprop and DMPA on growth of radish

The growth of radish in autoclaved sea sand was inhibited in a concentration-dependent manner by clomeprop and DMPA to a similar degree to that in non-autoclaved sea sand, where the growth was inhibited by DMPA to a slightly greater extent than by clomeprop (Fig 1). Figure 2 shows the effect of clomeprop and DMPA on the growth in soil. In non-autoclaved soil, inhibition was greater by DMPA than by clomeprop, as was also the case with sea sand. In autoclaved soil, however, growth was markedly inhibited by DMPA, as a function of concentration, but not by clomeprop even at the highest concentration. It is important to note that the phytotoxic activity of clomeprop was reduced more markedly in autoclaved soil than in non-autoclaved soil, while that of DMPA was similar under both soil conditions.

3.2 Adsorption and degradation of clomeprop and DMPA in soil

Table 1 shows the adsorption properties of clomeprop and DMPA on soil described by the Freundlich equation:[15]

$$X/m = K_f C^{1/n}$$

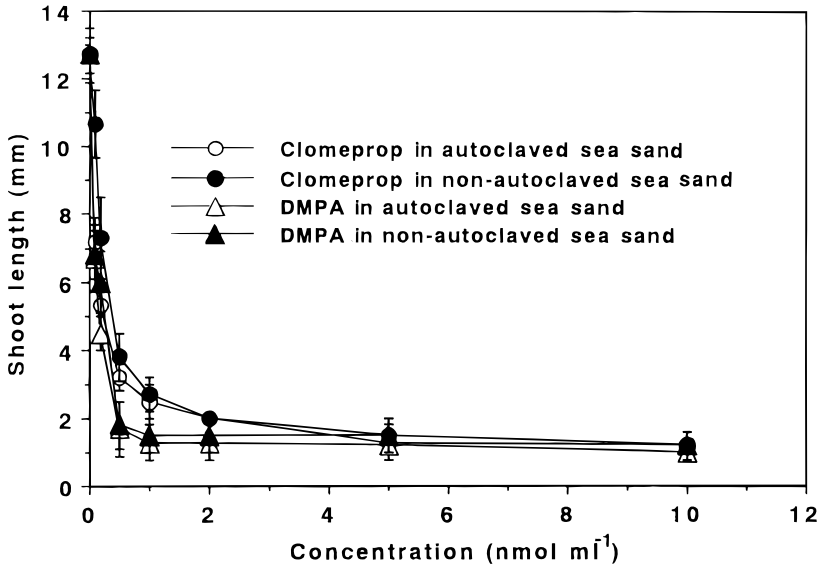

Figure 1. Effect of clomeprop and DMPA on shoot growth of radish seedlings in autoclaved and non-autoclaved sea sand. Vertical bars indicate standard errors.

where X/m is the amount of herbicide adsorbed (nmol g^{-1} dry soil) and C is the equilibrium concentration (nmol ml^{-1}); K_f and $1/n$ are empirical constants; K_f is the Freundlich coefficient (ml g^{-1} dry soil) and $1/n$ is a dimensionless parameter. The K_f values indicate that the adsorption of clomeprop on soil was much larger than that of DMPA, suggesting that K_f differed significantly between the herbicide and its metabolite, as in the case of atrazine,[16] imazethapyr[15] and bentazone.[17] These results indicate that the adsorption of clomeprop was largely controlled by soil organic matter, whereas the adsorption of DMPA depended mostly on the clay minerals and scarcely at all on the organic matter. Figure 3 shows the amount of chlomeprop and DMPA in the non-autoclaved soil at various time intervals after the application of clomeprop. At the onset, the concentration of clomeprop in soil water was the highest (0.21 nmol ml^{-1}) and the concentration of DMPA formed was found to be similar (0.23 nmol ml^{-1}) to that in soil water. The concentration of DMPA in soil water increased markedly with the decrease of the clomeprop concentration in soil water in a time-dependent manner. The amount of clomeprop adsorbed on the soil solid phase decreased with time and DMPA adsorption on soil solid phase occurred 72 h after the onset. This fact indicated that the DMPA increase was more marked in soil water than in the soil solid phase. The concentration of DMPA was much higher in soil water than

Table 1. Soil adsorption properties of clomeprop and DMPA

Chemical	*Kf*	*1/n*	r^2	K_{oc}
Clomeprop	16.78	0.69	0.91	2663
DMPA	0.54	0.87	0.87	85

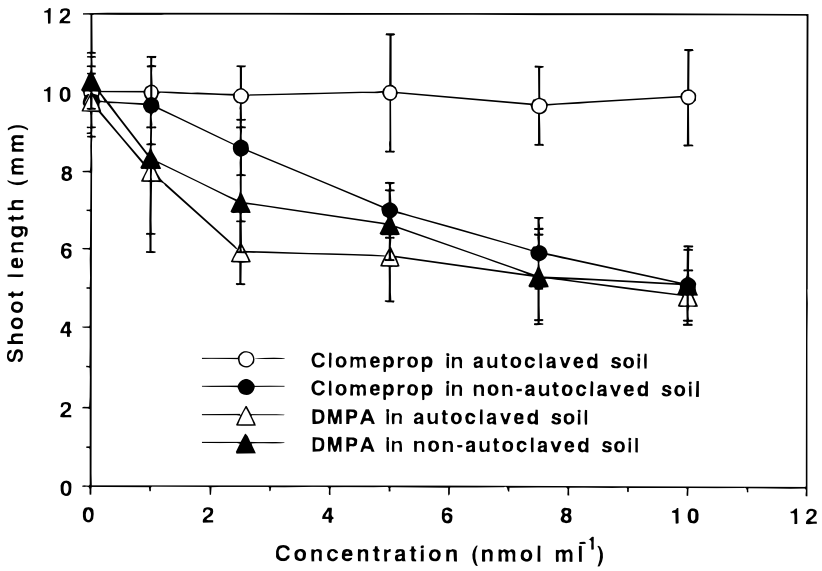

Figure 2. Effect of clomeprop and DMPA on shoot growth of radish seedlings in autoclaved and non-autoclaved soil. Vertical bars indicate standard errors.

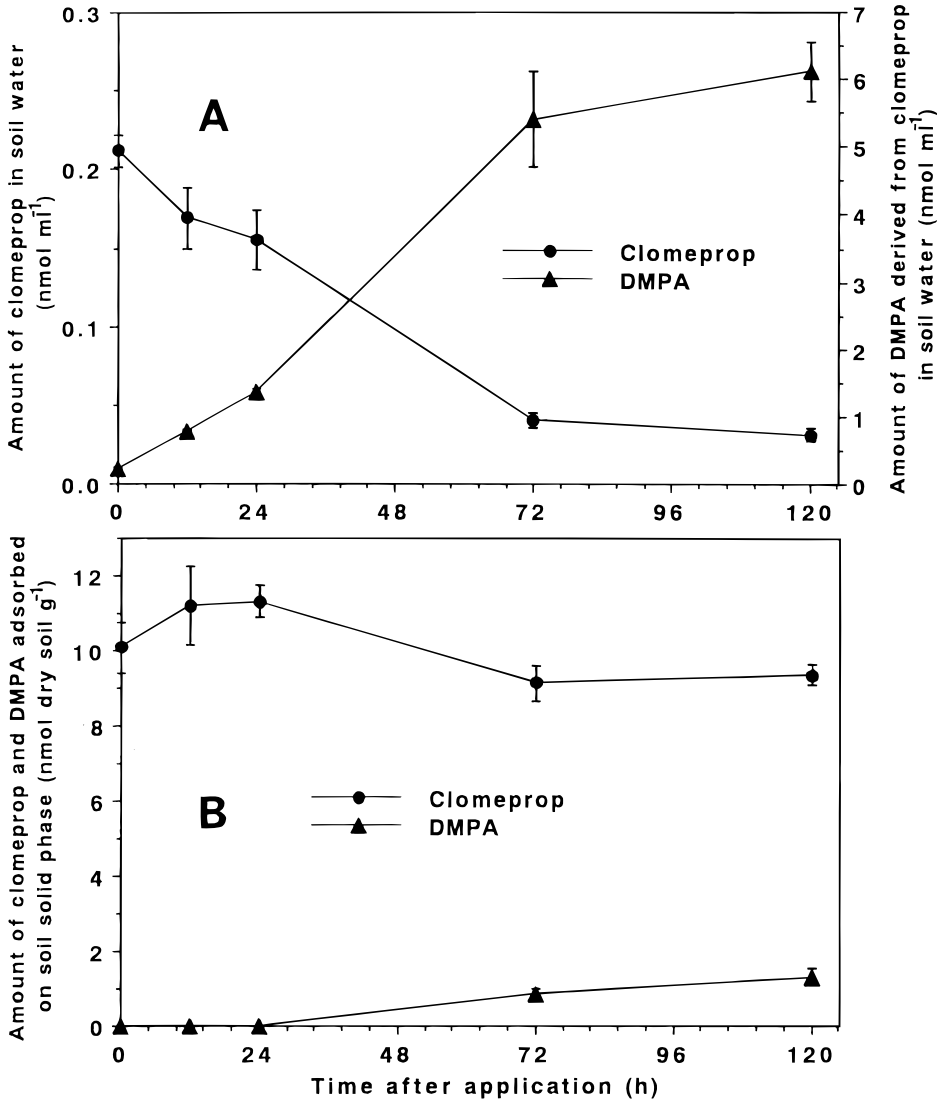

Figure 3. Time-course of the amount of clomeprop and DMPA (A) in soil water and (B) soil solid phase in Ryugasaki soil treated with clomeprop. Vertical bars indicate standard errors.

in soil solid, while that of clomeprop was higher in soil solid phase, with a good correlation with the soil adsorption properties (Table 1).

4 DISCUSSION

The similarity of the phytotoxic activity of clomeprop to that of DMPA in sea sand culture (Fig 1), and in water culture[3] suggested that clomeprop in the water present in the culture medium inhibits the growth to a similar extent to that of DMPA when the concentration of the herbicide was similar to that of DMPA in the solution. Therefore, the lower phytotoxic activity of clomeprop and DMPA in autoclaved soil (Fig 2) than in sea sand (Fig 1) might be attributed to the reduced concentration in soil water due to the adsorption on soil. The higher phytotoxic activity of clomeprop in non-autoclaved soil than in autoclaved soil suggested that the phytotoxic activity was not related to the amount of clomeprop itself in soil, because this was lower in non-autoclaved soil than in autoclaved soil, due to microbial degradation (data not shown). On the other hand, it was suggested that the lower phytotoxity of DMPA in non-autoclaved soil than in autoclaved soil was related to its metabolism in non-autoclaved soil. Our previous studies[10–13] revealed that the phytotoxic activity of herbicides in soil depended on the concentration in soil water. It is thus assumed that the lack of phytotoxic activity of clomeprop in autoclaved soil was due to the fact that its concentration in the soil water was insufficient to inhibit plant growth, due to its high adsorption on the soil solid phase. On the other hand, the marked activity of DMPA in autoclaved soil was associated with an adequate concentration in soil water due to its limited adsorption on the soil solid phase (Table 1, Fig 3). In non-autoclaved soil, the time-dependent decrease in clomeprop concentration with the corresponding increase in the DMPA concentration in soil water (Fig 3) suggested that plant growth inhibition in non-autoclaved soil treated with clomeprop depended on the DMPA

concentration in soil water but not on that of clomeprop. These findings may account for the reduction of the phytotoxity of clomeprop in autoclaved soil, as previously suggested. The decrease with time in the amount of clomeprop adsorbed on the soil solid phase (Fig 3) may be due to the desorption of clomeprop from the soil solid phase, which was controlled by the decreasing concentration in soil water due to microbial degradation to DMPA. It was suggested that the time-dependent increase of the concentration of DMPA in soil water and in the soil solid phase was associated with the more rapid hydrolysis of clomeprop to DMPA than the further metabolism of DMPA to other compounds in soil water. It is thus suggested that plant growth inhibition in soil treated with clomeprop was actually induced by the DMPA derived from the hydrolytic reaction of clomeprop in soil water. As a result, the increase of the DMPA led to phytotoxic activity in the soil water due to the lower adsorption on the soil solid phase. Further studies should be conducted to analyse the process, including the phytotoxic activity of clomeprop in paddy soils in relation to environmental factors such as water movement in soil, which may affect the concentration in soil water.

5 CONCLUSION

The lower phytotoxic activity of clomeprop in soil than in sea sand was attributed to a larger adsorption on the soil solid phase, whereas the slight reduction of the DMPA activity on plant growth in soil was due to its reduced adsorption on the soil solid phase. Phytotoxic activity of clomeprop in soil depends on the concentration of its hydrolysed metabolite DMPA in soil water but not on the concentration of clomeprop, itself. The concentration of DMPA in soil water is determined primarily by the degradation of clomeprop in soil by soil microbes, which is controlled by the adsorption–desorption equilibrium affected by microbial degradation of these chemicals in soil.

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